REMARKS

Claims 1, 2, 4, 9, 25, 26, 31, 39 and 43 have been amended to change "liquid wash culture medium" to "liquid culture medium." Claims 1, 25 and 39 have further been amended to indicate that this liquid culture medium "comprises nutrients and an osmoticum." Support for this latter amendment can be found in Example 1 in which the wash medium is liquid DCR_4. *See*, page 23, paragraph [00063]. DCR_4 comprises nutrients and an osmoticum as shown in Table 2. DCR_4 also comprises hormones. Support can also be found in Example 6 in which the wash medium is the maintenance medium of U.S. 5,565,355 without gelling agent. *See*, page 49, paragraph [0122]. The maintenance medium of the '355 patent comprises nutrients and an osmoticum. In addition, paragraph [0069] on page 24 indicates that the wash medium is a culture medium. Culture media are known in the art to contain nutrients and an osmoticum, and may optionally contain hormones. Consequently, the "liquid culture medium" has been defined in the claims to comprise nutrients and an osmoticum.

It is submitted that these amendments do not constitute new matter and their entry is requested.

The Examiner rejected claims 1-9, 11-43 and 45 under 35 U.S.C. § 112, first paragraph for failing to comply with the written description requirement. The Examiner contends that the term "liquid wash culture medium" constitutes new matter in the use of the word "culture." Applicants submit that the Examiner is in error in this rejection.

Although the language objected to by the Examiner does not appear *in haec verba* in the specification, Applicants submit that the specification contains an equivalent description of the claimed subject matter. Specifically, the specification describes the use of "DCR_4 liquid wash medium" in paragraph [0063] on page 23. This description is equivalent to a "liquid wash culture medium" because DCR_4 as shown in Table 2 is a culture medium. Furthermore, paragraph [0069] on page 24 states in part, "transferring the cells between the co-cultivation, wash, and post-wash culture media." Please note the use of the plural "media" and not the singular "medium." Thus, each of the terms "co-cultivation," "wash" and "post-wash" are types of culture media. To consider

otherwise is a nonsensical reading of this passage, because that would also imply that the word "co-cultivation" does not modify culture medium and thus must not be a culture medium. However, the specification teaches otherwise, as it does for the wash culture medium. Furthermore, paragraph [0122] on page 49 states in part, "wash medium identical to the maintenance medium except that gelling agents were omitted." Thus, since the maintenance medium is a culture medium, this wash medium is a liquid culture medium. Finally, paragraph [0031] on page 9 indicates that the cells are washed with a wash medium that is a liquid medium. This passage in combination with the previously cited passages clearly provides support for the term "liquid wash culture medium." As demonstrated above, although the specification does not contain a *in haec verba* description of the claimed invention, it does contain an equivalent description of the claimed invention. Such a description is sufficient. *See, Lockwood v. American Airlines Inc.*, 107 F.3d 1565, 41 USPQ2d 1961; *Eiselstein v. Frank*, 52 F.3d 1035, 34 USPQ2d 1467 (Fed. Cir. 1995).

Although Applicants believe that the specification contains a written description of the claimed invention., they have nevertheless amended the claims to specify that the cells are washed with a "liquid culture medium," which is fully supported by the specification as demonstrated above.

In view of the above amendments and remarks, it is submitted that the claimed invention is described in the specification. Withdrawal of this rejection is requested.

The Examiner rejected claims 1-9, 11-43 and 45 under 35 U.S.C. § 112, second paragraph as being indefinite for the use of the term "liquid wash culture medium." As described above, the objected term has been amended to read "liquid culture medium" which has further been defined to comprise nutrients and an osmoticum. The liquid culture media described in the Examples comprise nutrients and an osmoticum. Futhermore, it is well known in the tissue culture art that culture media contain nutrients and an osmoticum. Also, as discussed above, the specification, especially in paragraph [0069] on page 24 describes transferring the cells from a wash culture medium, the only logical reading of the language, "co-cultivation, wash, and post-wash culture media" in view of the plural usage of "media." Each of the specified items is a culture medium, hence the use of the plural term "media."

Definiteness is determined with reference to a person of ordinary skill in the art. *Miles Laboratories, Inc. v. Shandon Inc.*, 997 F.2d 870, 875, 27 U.S.P.Q.2d 1123, 1126 (Fed. Cir. 1993), *cert. denied*, 510 U.S. 1100 (1994) ("The test for definiteness is whether one skilled in the art would understand the bounds of the claim when read in light of the specification."); *In re Warmerdam*, 33 F.3d 1354, 1361, 31 U.S.P.Q.2d 1754, 1759 (Fed. Cir. 1994) ("The legal standard for definiteness is whether a claim reasonably apprises those of skill in the art of its scope."). Applicants submit that the term "liquid culture medium" with respect to the wash medium is definite because a skilled artisan, the person to whom the claims are directed, understands the metes and bounds of the term "liquid culture medium" when read in light of the specification.

Although Applicants believe that the term "liquid culture medium" is definite to a skilled artisan, the claims have further been amended to specify that the liquid culture medium comprises nutrients and an osmoticum. Applicants note that this amendment could not have been made previously because the Examiner first made the rejection in the present Office Action. Thus, Applicants submit that the metes and bounds of the claimed invention are clear and definite to skilled artisan. Withdrawal of this rejection is requested.

The Examiner rejected claims 1-9, 11-43 and 45-62 under 35 U.S.C. § 103(a) as being unpatentable over Levee et al. (*Molecular Breeding* 5:429-440, 1999). The Examiner stated that this rejection is maintained for the reasons of record set forth in the Office Action mailed 8 April 2004. It is submitted that the claimed invention is not obvious from the teachings of the cited art.

In accordance with the claimed invention, enhanced transformation and regeneration of transformed embryonic hard pine tissue is accomplished by minimizing damage to cells subsequent to *Agrobacterium* infection. It was found by the inventors that one technique to minimize damage to cells was to wash cells following *Agrobacterium* infection with a liquid culture medium, a limitation found in the claims. This feature is disclosed in the present specification as detailed above. Damage to pine cells of the subgenus *Pinus* is minimized by using a liquid culture medium to wash the pines cells subsequent to *Agrobacterium* infection. As discussed in the previous Amendments, distilled water could not be used to wash the pine cells, but any liquid culture medium

could be used. Levee et al. uses distilled water to wash pine cells of the subgenus *Strobus*, i.e., soft pines, following *Agrobacterium* infection.

In the previous Amendment filed on 9 August 2004, Applicants had argued that Levee et al. disclosed washing cells with water and did not disclose washing cells with a liquid wash culture medium. Applicants further argued that Levee et al. disclosed transformation and regeneration of soft pine and did not disclose the transformation and regeneration of hard pines. In response to these arguments, the Examiner stated on page 6 of the present Office Action that "it is noted that features upon which Applicants rely (i.e., a washing cells with DCR4 culture medium or any culture medium comprising nutrients, hormones and an osmoticum) are not recited in the rejected claim(s)." It is submitted that the Examiner is incorrect in this conclusion. In addition, the Examiner did not address the issue of hard pines versus soft pines, which in itself is sufficient to obviate the obviousness rejection.

As detailed in the present Amendment and in the Amendment filed on 9 August 2004, the specification fully describes the use of a liquid wash culture medium for washing the cells following *Agrobacterium* transformation. This language is especially described in paragraph [0069] which describes the use of support membranes for transferring cells between "the co-cultivation, wash, and post-wash culture media." As set forth above, the proper reading of this language is "the co-cultivation culture medium, wash culture medium and post-wash culture medium." The specification describes the co-cultivation of pine cells with *Agrobacterium*. This co-cultivation is accomplished using a culture medium. Thus, the use of the language "co-cultivation" in paragraph [0069] modifies the term "culture media" as per customary English grammatical usage. In addition, the specification describes washing the cells with a liquid culture medium as evidenced in Examples 1 and 6. Thus, the use of the language "wash" in paragraph [0069] also modifies the term "culture media" as per customary English grammatical usage, and the wash culture medium is a liquid wash culture medium as shown in the Examples and as stated in paragraph [0031], i.e., liquid wash medium. Thus, it is submitted that one of the features upon which Applicants rely, namely washing cells with a liquid wash culture medium, was recited in the rejected claims.

As stated above, although Applicants believe that the term "liquid wash culture medium" is described in the specification, the claims have been amended so that this term now reads "liquid culture medium." The "liquid culture medium" which is used to wash the transformed pine cells is therefore set forth in the claims, as was the original term (liquid wash culture medium). Thus, it is submitted that the Examiner is in error in his conclusion that the claims do not recite a feature upon which Applicants rely. Consequently, the claims contain the recited limitation and it does not need to be read into the claims from the specification.

Furthermore, as detailed above, and in response to the indefiniteness rejection (although they do not believe that it was necessary), Applicants have specified that the "liquid culture medium" used for washing the transformed pine cells comprises nutrients and an osmoticum. Thus, one of the features upon which Applicants rely has been amended to recite "washing cells with a liquid culture medium comprising nutrients and an osmoticum." This feature is not disclosed or suggested in Levee et al. which discloses washing cells with distilled water.

In summary, the claims recite the limitation upon which Applicants rely, i.e., the claims recite that the cells are washed with "a liquid culture medium comprising nutrients and an osmoticum." There is no suggestion in Levee et al. to use a liquid culture medium and no suggestion that use of a liquid culture medium would result in an enhanced transformation and regeneration of transformed embryonic tissue of hard pines. Thus, it is submitted that the claimed invention is not obvious from the teachings of Levee et al.

In addition to the limitation concerning the use of a liquid wash medium for washing the cells, Applicants also rely upon the limitation, specifically recited in the claims, that the pine cells are "pine cells of the subgenus *Pinus*." Pines of the subgenus *Pinus* are hard pines, whereas pines of the subgenus *Strobus* (the pine of Levee et al.) are soft pines. *See*, e.g., the attached *Pinus* description which shows that the subgenus *Pinus* are hard pines, whereas the subgenus *Strobus* are soft pines. Thus, the *Agrobacterium* transformation disclosed in Levee et al. is transformation of a soft pine and not of a hard pine, the latter being a limitation of the claims. As detailed in the Amendment filed 9 August 2004 and supported by the Rule 132 Declarations of Dr. Connett-

Porceddu, Dr. Becwar, Dr. Canavera and Dr. Mann there are well known differences between hard pines and soft pines and there was no reasonable expectation that transformation and regeneration methods that worked with one group of pines would work with the other group of pines. Because of these facts, it is submitted that the claimed invention is not obvious from Levee et al.

To recap the salient points from the Amendment filed 9 August 2004 and the Rule 132 Declarations, Applicants reiterate the following points concerning the nonobviousness of the transformation of hard pines by *Agrobacterium*.

Levee et al. discloses the transformation and regeneration of pine of the subgenus *Strobus* which, according to this reference, "is the first work on genetic transformation on **this pine species** as well as the first report of successful stable genetic transformation of **a pine species** using a disarmed strain of *A. tumefaciens*". (See page 36, first paragraph of Discussion, emphasis added). Levee et al. does not disclose the transformation and regeneration of pine of the subgenus *Pinus*. The amended claims are clearly directed to pine cells of the *Pinus* subgenus. It is well known to those skilled in the art that somatic embryogenesis systems for soft pines are different from those for hard pines. It is not insignificant that Levee et al. utilized a soft pine which is more easily regenerated than hard pines. Although the Examiner cited art showing transformation and regeneration of soft pine, he has not cited any art showing transformation and regeneration of hard pines as claimed. Furthermore, it is submitted that there has been no reports in the literature of the regeneration of plants following stable transformation by *Agrobacterium* of embryogenic cultures of any pines of the *Pinus* subgenus.

Applicants have previously discussed differences between hard and soft pines and the prior inability to regenerate transformed pine tissue of pines of the subgenus *Pinus*, i.e., hard pines, in commercially valuable quantities. One feature of the invention which enables the enhanced transformation and the regeneration of transformed embryonic hard pine tissue is the minimization of damage to the pine cells which is accomplished by using a liquid culture medium as opposed to using water to wash cells following *Agrobacterium* infection or cocultivation of embryonic hard pine tissue with *Agrobacterium*. This feature of the invention is found in the claims. The differences

between hard and soft pines leads to the unobvious nature of plant transformation and regeneration in these species.

Applicants submit that the Amendment filed on 9 August 2004, in combination with the Rule 132 Declarations filed therewith, clearly demonstrated that there are, and were at the time of the present invention, well known differences between hard and soft pines. That Amendment and the Rule 132 Declarations further demonstrated the unobvious nature of plant transformation and regeneration in these species. These differences (i.e., the differences between hard and soft pines and the differences in plant transformation and regeneration in hard pines and soft pines) present evidence that transformation and regeneration in one conifer species, such as soft pines, could not be applied to another conifer species, such as hard pines. The comments and evidence demonstrating the unobvious nature of the present invention are incorporated herein by reference. The Examiner has not cited any evidence that would rebut the evidence previously presented.

For example, the Rule 132 Declaration of Dr. Connett-Porceddu establishes that:

(a) there were known differences between hard pines and soft pines (Paragraph 6);

(b) hard pines could be transformed and regenerated to produce transgenic hard pine plants using the method claimed in the present application (Paragraph 6);

(c) the present invention allowed for the first time *Agrobacterium*-transformation followed by regeneration of transgenic hard pine plants at a significant frequency (Paragraph 6);

(d) although the cited prior art (Levee et al.) discloses the transformation and regeneration of pine of the subgenus *Strobus*, this prior art does not show the transformation and regeneration of pines of the subgenus *Pinus*, and a skilled artisan would not expect that the method for soft pines (subgenus *Strobus*) could be used or routinely modified for use with hard pines (subgenus *Pinus*) (Paragraph 7);

(e) it was known at the time of the present invention that there were differences between soft pines and hard pines as seen in transformation and regeneration methods for soft pines and hard pines, such that there were no expectation of success with respect to the transformation of hard or soft pines on the basis of the other (Paragraphs 8-10);

(f) there had been no reports of the regeneration of transgenic plants of hard pines (i.e., pines of the subgenus *Pinus*) prior to the present invention and any reports at all concerning regeneration of transgenic hard pines demonstrated that regeneration was not achieved (Paragraph 11);

(g) it is noteworthy that the cited Levee et al. prior art did not discuss at all the regeneration of transgenic plants of hard pine which is the most economic species of conifers (Paragraph 12);

(h) there have no reports of the application of the method of Levee et al. to the regeneration of transgenic hard pines and in fact, Levee himself has not continued use of the disclosed method for even soft pines, as well as the fact that the assignee of the present application has tried to use or modify the method described by Levee et al. for the regeneration of transgenic hard pine but has not been successful (Paragraph 12); and

(i) experiments had been underway at the assignee of the present application for more than 10 years to adapt systems for regeneration hard pines and for transforming and regenerating transformed hard pines and that somatic embryogenesis systems had been developed which worked well with hard pines, but not with transgenic hard pines (Paragraph 13).

The inability to adapt systems developed for transgenic soft pines to transgenic hard pines is further evidence of the differences between soft pines and hard pines and is evidence of no expectation of success in the art for using systems for transgenic soft pines for regenerating transgenic hard pines. *See* Paragraph 13 of the Connett-Porceddu Declaration. Since (a) a person of ordinary skill in the art knew that there were differences between soft pines (subgenus *Strobus*) and hard pines (subgenus *Pinus*) with respect to tissue culture, regeneration and transformation and (b) there was a lack of application of methods between the soft and hard pines, there was no expectation of success in the art for regenerating transgenic hard pines on the basis of a single report for the regeneration of transgenic soft pines. *See* Paragraph 14 of the Connett-Porceddu Declaration. Several of these facts are also supported by the Rule 132 Declarations of Dr. Becwar and Dr. Canavera as described in detail in the Amendment filed 9 August 2004.

Also, the Rule 132 Declaration of Dr. Canavera establishes that:

(a) there was a long-felt need to develop (i) improved methods of *Agrobacterium* transformation of hard pines and improved selection of transformed tissue and (ii) methods to regenerate *Agrobacterium*-transformed hard pines, which was not satisfied by transformation of other conifers (Paragraph 7);

(b) the method for hard pine transformation and regeneration described and claimed in the present application and the method for selection of transgenic Southern yellow pine tissue described and claimed in companion application Serial No. 09/973,089 are the first methods that achieved reliable and efficient regeneration of transgenic hard pine plants (Paragraph 8);

(c) the reliable and efficient regeneration of transgenic hard pine results directly from the methods described and claimed in these applications (Paragraph 8);

(d) these methods are sufficiently robust to fill the long-felt need because the methods have been shown to be valid for a wide variety of genotypes of hard pines, a result which had not been achieved without these methods (Paragraph 8); and

(e) a method such as one used by the Canadian Forest Service (Levee et al.) that is not able to be used for multiple species did not meet the long felt need for the regeneration of transgenic hard pines, whereas the method described and claimed in Serial No. 09/973,088 does satisfy the long-held need (Paragraph 9).

In addition, the Rule 132 Declaration of Dr. Mann confirms the long felt need described by Dr. Canavera and establishes the commercial success of the present invention (Paragraphs 6-11).

In summary, the cited prior art does not disclose or suggest using a liquid culture medium comprising nutrients and an osmoticum for washing pine cells of the *Pinus* subgenus following incubation with *Agrobacterium*. The use of a the liquid culture medium minimizes damage to the pine cells, which enables the regeneration of transgenic hard pines. Also, the cited prior art does not disclose or suggest the regeneration of transgenic plants of pine of the genus *Pinus* subgenus *Pinus*. The evidence presented in the prior art and in the Rule 132 Declarations demonstrate that there is no expectation of success in this art, and more particularly, that there is no expectation that methods useful for one conifer species, such as the soft pine of Levee et al., can be used for another conifer

species, such as the claimed hard pines. In addition, the Examiner has not cited any evidence that would rebut this evidence. Thus, it is submitted that Levee et al. does not render the claimed invention obvious. Withdrawal of this rejection is requested.

In view of the above amendments and remarks, and in conjunction with the remarks made in the previous amendments and previously filed Rule 132 Declarations, it is believed that the claims satisfy the requirements of the patent statutes and are patentable over the prior art. Reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

ROTHWELL, FIGG, ERNST & MANBECK, p.c.

By _____

Jeffrey L. Ihnen
Registration No. 28,957
Attorney for Applicant
1425 K Street, N.W., Suite 800
Washington, D.C. 20005
phone: 202-783-6040
fax: 202-783-6031

Attachment: "*Pinus* description,"from the Gymnosperm Database,
URL: http://www.conifers.org/pi/pin/index.htm,
edited by C.J. Earle, last modified on 8 Dec. 2004.

S:\Data\Clients\2411\2411-110.amend4.wpd